SEQUENCE LISTING

- <110> Ding, Jeak Ling Ho, Bow National University of Singapore
- <120> Sushi Peptide Multimer
- <130> 040184-000400US
- <140> US 10/563,551
- <141> 2006-01-04
- <150> CA 2,432,972
- <151> 2003-07-04
- <150> WO PCT/SG04/00194
- <151> 2004-07-02
- <160> 11
- <170> PatentIn Ver. 2.1
- <210> 1
- <211> 34
- <212> PRT
- <213> Artificial Sequence
- <220×
- <223> Description of Artificial Sequence:synthetic
 34-mer Sushi-3 peptide (S3 peptide), residues
 268-301 of Factor C, Sushi3 domain, LPS-binding
 motif
- <400> 1
- His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln 1 5 10 15
- Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe 20 25 30

Leu Met

- <210> 2
- <211> 34
- <212> PRT
- <213> Artificial Sequence
- <220>
- <223> Description of Artificial Sequence:synthetic 34-mer Sushi-3delta peptide (S3delta peptide)
- <400> 2
- His Ala Glu His Lys Val Lys Ile Lys Val Lys Gln Lys Tyr Gly Gln 1 5 10 15
- Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe 20 25 30

Leu Met

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<210> 3
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      motif S3 PCR amplification forward primer
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<210> 4
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<223> Description of Artificial Sequence: LPS-binding
      motif S3 PCR amplification reverse primer
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<211> 3448
<212> DNA
<213> Carcinoscorpius rotundicauda
<223> Factor C cDNA
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<221> CDS
<222> (18)..(3077)
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gtt cta ggg cta cta gcc caa aaa atg cgc cca gtt cag tcc aaa gga
                                                                    98
Val Leu Gly Leu Leu Ala Gln Lys Met Arg Pro Val Gln Ser Lys Gly
                                 20
gta gat cta ggc ttg tgt gat gaa acg agg ttc gag tgt aag tgt ggc
                                                                    146
Val Asp Leu Gly Leu Cys Asp Glu Thr Arg Phe Glu Cys Lys Cys Gly
gat cca ggc tat gtg ttc aac att cca gtg aaa caa tgt aca tac ttt
                                                                    194
Asp Pro Gly Tyr Val Phe Asn Ile Pro Val Lys Gln Cys Thr Tyr Phe
tat cga tgg agg ccg tat tgt aaa cca tgt gat gac ctg gag gct aag
                                                                    242
Tyr Arg Trp Arg Pro Tyr Cys Lys Pro Cys Asp Asp Leu Glu Ala Lys
60
                     65
                                          70
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gat att tgt cca aag Asp Ile Cys Pro Lys 80	Tyr Lys Arg C			290
gat agt tgt gtt act Asp Ser Cys Val Thi 95	Cys Pro Pro A			338
ggt gaa tgt cag tgt Gly Glu Cys Gln Cys 110			Arg Thr Gly	386
gct tgt gca tgt cgt Ala Cys Ala Cys Arg 125				434
aaa ggt tgt cct ctt Lys Gly Cys Pro Let 140				482
aat cca cca gat aat Asn Pro Pro Asp Asr 160	Pro Gln Thr I			530
ttc aag ctt aag ggt Phe Lys Leu Lys Gly 175	Met Ala Arg I			578
tgg agt aac ttt cca Trp Ser Asn Phe Pro 190			Met Val Ser	626 :
tct cca gaa cat ggg Ser Pro Glu His Gly 205				674
ggg gct act tta cgg Gly Ala Thr Leu Arg 220		-	2 22	722
caa gaa aca tta acc Gln Glu Thr Leu Thr 240	Cys Gln Gly A			770
cca caa tgt aag aad Pro Gln Cys Lys Asr 255	Leu Val Phe C			818
cat gct gaa cac aag His Ala Glu His Lys 270	_		Tyr Gly Gln	866
ttt cct caa ggc act Phe Pro Gln Gly Thr 285				914
ttg atg ggt ttt gad Leu Met Gly Phe Asg 300				962

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								gtg Val								1010
								gat Asp 340								1058
			_					ttg Leu								1106
								tca Ser		_	_	_	_			1154
								gga Gly								1202
						_		agt Ser	_	_					_	1250
	_		_	_		_		agt Ser 420		_		_		_	_	1298
.₹								tgt Cys								1346
3	-			_		_		tca Ser		_	_	_		_	_	1394
								gct Ala								1442
		_	_			_	_	act Thr				_				1490
								cac His 500								1538
								agt Ser								1586
								cct Pro								1634
								cag Gln								1682

				tca Ser										1730			
				560				565				570		1850			
				tgc Cys										1778			
				caa Gln	_	_				_				1826			
		_	_	gag Glu	_				_		_		_	1874			
	_			aat Asn			_	_			_	_		1922			
				tgc Cys 640										1970			
٠.				cca Pro										2018			
				ttg Leu										2066	*•		
				aaa Lys										2114			
				aaa Lys										2162			
				tgc Cys 720										2210			
				gac Asp										2258			
				tgt Cys										2306			
				tct Ser										2354			
				ctt Leu										2402			

gga to Gly Se															2450
acc ta Thr Ty	ac tct /r Ser	gct Ala 815	act Thr	gct Ala	gag Glu	att Ile	att Ile 820	gac Asp	ccc Pro	aat Asn	cag Gln	ttt Phe 825	aaa Lys	atg Met	2498
tat ct Tyr Le		Lys													2546
caa gt Gln Va 84	al Arg														2594
ggc aa Gly As 860															2642
act tt Thr Le															2690
aca ag Thr Ar									-						2738
ggt tt Gly Le		Glu													2786
cta co Leu Pr 92	o Val														2834
gac tt Asp Le 940										_	_				2882
aag gg Lys Gl	ga cgt y Arg	tat Tyr	gat Asp 960	gcc Ala	tgc Cys	agt Ser	gjà aaa	gac Asp 965	agt Ser	gga Gly	gga Gly	cct Pro	tta Leu 970	gtg Val	2930
ttt go Phe Al															2978
gtc ag Val Se										Ala					3026
ggc tt Gly Ph 100	e Thr			Asn					\mathtt{Trp}						3074
tga aa	ctgat	cta a	aatat	ttta	aa go	catgo	gttat	aaa	cgto	ttg	tttc	cctat	ta		3127
1020															

ttgctttact agtttaaccc ataagaaggt taactgggta aggcacaagg atcattgttt 3187

ctgtttgttt ttacaaatgg ttattttagt cagtgaatga gaatagtatc cattgaagac 3247
tgttaccttt tattctacct ttttatatta ctatgtaagt atttgggata tcttctacac 3307
atgaaaattc tgtcatttta ccataaattt ggtttctggt gtgtgctaag tccaccagta 3367
gagaacgatg taattttcac tagcacatga aataaatata gaacaaatct attataaact 3427
accttaaaaa aaaaaaaaaa a

<210> 6 <211> 1019 <212> PRT <213> Carcinoscorpius rotundicauda <220> <223> Factor C <400> 6 Met Val Leu Ala Ser Phe Leu Val Ser Gly Leu Val Leu Gly Leu Leu 10 Ala Gln Lys Met Arg Pro Val Gln Ser Lys Gly Val Asp Leu Gly Leu 2.0 25 Cys Asp Glu Thr Arg Phe Glu Cys Lys Cys Gly Asp Pro Gly Tyr Val 40 Phe Asn Ile Pro Val Lys Gln Cys Thr Tyr Phe Tyr Arg Trp Arg Pro 55 Tyr Cys Lys Pro Cys Asp Asp Leu Glu Ala Lys Asp Ile Cys Pro Lys 70 75 Tyr Lys Arg Cys Gln Glu Cys Lys Ala Gly Leu Asp Ser Cys Val Thr 85 90 Cys Pro Pro Asn Lys Tyr Gly Thr Trp Cys Ser Gly Glu Cys Gln Cys 105 Lys Asn Gly Gly Ile Cys Asp Gln Arg Thr Gly Ala Cys Ala Cys Arg 125 Asp Arg Tyr Glu Gly Val His Cys Glu Ile Leu Lys Gly Cys Pro Leu 135 Leu Pro Ser Asp Ser Gln Val Gln Glu Val Arg Asn Pro Pro Asp Asn 155 Pro Gln Thr Ile Asp Tyr Ser Cys Ser Pro Gly Phe Lys Leu Lys Gly 170 Met Ala Arg Ile Ser Cys Leu Pro Asn Gly Gln Trp Ser Asn Phe Pro 185 Pro Lys Cys Ile Arg Glu Cys Ala Met Val Ser Ser Pro Glu His Gly 200 Lys Val Asn Ala Leu Ser Gly Asp Met Ile Glu Gly Ala Thr Leu Arg 215 220 Phe Ser Cys Asp Ser Pro Tyr Tyr Leu Ile Gly Gln Glu Thr Leu Thr 230 235 Cys Gln Gly Asn Gly Gln Trp Asn Gly Gln Ile Pro Gln Cys Lys Asn 245 250 Leu Val Phe Cys Pro Asp Leu Asp Pro Val Asn His Ala Glu His Lys 265 Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr 280 Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Gly Phe Asp 295 300 Thr Leu Lys Cys Asn Pro Asp Gly Ser Trp Ser Gly Ser Gln Pro Ser

315

310

Cys Val Lys Val Ala Asp Arg Glu Val Asp Cys Asp Ser Lys Ala Val Asp Phe Leu Asp Asp Val Gly Glu Pro Val Arg Ile His Cys Pro Ala Gly Cys Ser Leu Thr Ala Gly Thr Val Trp Gly Thr Ala Ile Tyr His Glu Leu Ser Ser Val Cys Arg Ala Ala Ile His Ala Gly Lys Leu Pro Asn Ser Gly Gly Ala Val His Val Val Asn Asn Gly Pro Tyr Ser Asp Phe Leu Gly Ser Asp Leu Asn Gly Ile Lys Ser Glu Glu Leu Lys Ser Leu Ala Arg Ser Phe Arg Phe Asp Tyr Val Arg Ser Ser Thr Ala Gly Lys Ser Gly Cys Pro Asp Gly Trp Phe Glu Val Asp Glu Asn Cys Val Tyr Val Thr Ser Lys Gln Arg Ala Trp Glu Arg Ala Gln Gly Val Cys Thr Asn Met Ala Ala Arg Leu Ala Val Leu Asp Lys Asp Val Ile Pro Asn Ser Leu Thr Glu Thr Leu Arg Gly Lys Gly Leu Thr Thr Trp Ile Gly Leu His Arg Leu Asp Ala Glu Lys Pro Phe Ile Trp Glu Leu Met Asp Arg Ser Asn Val Val Leu Asn Asp Asn Leu Thr Phe Trp Ala Ser Gly Glu Pro Gly Asn Glu Thr Asn Cys Val Tyr Met Asp Ile Gln Asp Gln Leu Gln Ser Val Trp Lys Thr Lys Ser Cys Phe Gln Pro Ser Ser Phe Ala Cys Met Met Asp Leu Ser Asp Arg Asn Lys Ala Lys Cys Asp Asp Pro Gly Ser Leu Glu Asn Gly His Ala Thr Leu His Gly Gln Ser Ile Asp Gly Phe Tyr Ala Gly Ser Ser Ile Arg Tyr Ser Cys Glu Val Leu His Tyr Leu Ser Gly Thr Glu Thr Val Thr Cys Thr Thr Asn Gly Thr Trp Ser Ala Pro Lys Pro Arg Cys Ile Lys Val Ile Thr Cys Gln Asn Pro Pro Val Pro Ser Tyr Gly Ser Val Glu Ile Lys Pro Pro Ser Arg Thr Asn Ser Ile Ser Arg Val Gly Ser Pro Phe Leu Arg Leu Pro Arg Leu Pro Leu Pro Leu Ala Arg Ala Ala Lys Pro Pro Pro Lys Pro Arg Ser Ser Gln Pro Ser Thr Val Asp Leu Ala Ser Lys Val Lys Leu Pro Glu Gly His Tyr Arg Val Gly Ser Arg Ala Ile Tyr Thr Cys Glu Ser Arg Tyr Tyr Glu Leu Leu Gly Ser Gln Gly Arg Arg Cys Asp Ser Asn Gly Asn Trp Ser Gly Arg Pro Ala Ser Cys Ile Pro Val Cys Gly Arg Ser Asp Ser Pro Arg Ser Pro Phe Ile Trp Asn Gly Asn Ser Thr Glu Ile Gly Gln Trp Pro Trp Gln Ala Gly Ile Ser Arg Trp Leu Ala Asp His Asn Met Trp Phe Leu Gln Cys Gly Gly Ser Leu Leu Asn

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Glu Lys Trp Ile Val Thr Ala Ala His Cys Val Thr Tyr Ser Ala Thr
               805
                                   810
Ala Glu Ile Ile Asp Pro Asn Gln Phe Lys Met Tyr Leu Gly Lys Tyr
           820
                           825
Tyr Arg Asp Asp Ser Arg Asp Asp Tyr Val Gln Val Arg Glu Ala
                          840
Leu Glu Ile His Val Asn Pro Asn Tyr Asp Pro Gly Asn Leu Asn Phe
                       855
                                           860
Asp Ile Ala Leu Ile Gln Leu Lys Thr Pro Val Thr Leu Thr Thr Arg
                   870
                                      875
Val Gln Pro Ile Cys Leu Pro Thr Asp Ile Thr Thr Arg Glu His Leu
               885
                                  890
Lys Glu Gly Thr Leu Ala Val Val Thr Gly Trp Gly Leu Asn Glu Asn
           900
                               905
Asn Thr Tyr Ser Glu Thr Ile Gln Gln Ala Val Leu Pro Val Val Ala
                           920
       915
                                              925
Ala Ser Thr Cys Glu Glu Gly Tyr Lys Glu Ala Asp Leu Pro Leu Thr
                       935
                                          940
Val Thr Glu Asn Met Phe Cys Ala Gly Tyr Lys Lys Gly Arg Tyr Asp
                   950
                                      955
Ala Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Phe Ala Asp Asp Ser
               965
                                  970
Arg Thr Glu Arg Arg Trp Val Leu Glu Gly Ile Val Ser Trp Gly Ser
           980
                               985
Pro Ser Gly Cys Gly Lys Ala Asn Gln Tyr Gly Gly Phe Thr Lys Val
       995 1000
Asn Val Phe Leu Ser Trp Ile Arg Gln Phe Ile
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                      1015
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<210> 7
<211> 36
<212> PRT
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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:recombinant S3 monomer (rS3-1mer) peptide, with additional Pro and Asp at ends from acid cleavage of rS3-4mer DP linker

<400> 7

Pro His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln Lys Tyr Gly
1 5 10 15

Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr
20 25 30

Phe Leu Met Asp 35

<210> 8 <211> 468 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence:recombinant S3 tetramer (rS3-4mer) tandem repeat peptide with acid cleavable DP linker between S3 units <220> <221> CDS <222> (1)..(468) <223> rS3-4mer <400> 8 ccc cag gat ccc cat gct gaa cac aag gtt aaa att ggt gtg gaa caa 48 Pro Gln Asp Pro His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln aaa tat ggt cag ttt cct caa ggc act gaa gtg acc tat acg tgt tcg 96 Lys Tyr Gly Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser 20 25 ggt aac tac ttc ttg atg gac ccc cag gat ccc cat gct gaa cac aag 144 Gly Asn Tyr Phe Leu Met Asp Pro Gln Asp Pro His Ala Glu His Lys 35 gtt aaa att ggt gtg gaa caa aaa tat ggt cag ttt cct caa ggc act 192 Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr 50 55 gaa gtg acc tat acg tgt tcg ggt aac tac ttc ttg atg gac ccc cag Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Asp Pro Gln 70 gat ccc cat gct gaa cac aag gtt aaa att ggt gtg gaa caa aaa tat Asp Pro His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln Lys Tyr ggt cag ttt cct caa ggc act gaa gtg acc tat acg tgt tcg ggt aac 336 Gly Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn 100 tac ttc ttg atg gac ccc cag gat ccc cat gct gaa cac aag gtt aaa 384 Tyr Phe Leu Met Asp Pro Gln Asp Pro His Ala Glu His Lys Val Lys 115 120 att ggt gtg gaa caa aaa tat ggt cag ttt cct caa ggc act gaa gtg 432 Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr Glu Val 130 135 acc tat acg tgt tcg ggt aac tac ttc ttg atg gac 468 Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Asp 145 150 <210> 9 <211> 156 <212> PRT <213> Artificial Sequence <220> <223> Description of Artificial Sequence:recombinant S3 tetramer (rS3-4mer) tandem repeat peptide with

acid cleavable DP linkerbetween S3 units

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Lys Tyr Gly Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser
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Gly Asn Tyr Phe Leu Met Asp Pro Gln Asp Pro His Ala Glu His Lys
                             40
Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr
Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Asp Pro Gln
Asp Pro His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln Lys Tyr
Gly Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn
                                105
Tyr Phe Leu Met Asp Pro Gln Asp Pro His Ala Glu His Lys Val Lys
       115
Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr Glu Val
                        135
Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Asp
                    150
<210> 10
<211> 4
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:amino acids
     used to link S3 multimer to remainder of
     polypeptide
<400> 10
Ile Glu Gly Arg
<210> 11
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<213> Artificial Sequence
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<220>
<221> MOD RES
<222> (1)..(7)
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<223> Xaa = any amino acid

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<220>
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<222> (4)..(5)
<223> Asp at positions 4 and 5 may be present or absent
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Xaa Asp Asp Asp Asp Lys Xaa
1 5
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